Third Status Report of Research on

"Effects of Plant Growth Hormones on Plant Development in the Absence of Gravitational Effects"

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**UNPUBLISHED PRELIMINARY DATA**

The primary objective of our basic research continues to be the assembly of facts about the role and mechanics of gravity in maintaining an erect axis in a leafy plant while the leaves and branches grow at characteristic angles. We have considerable data to support our thesis that the growth curvature of an axis in the absence of the effects of gravity, as on a horizontal clinostat, is due to uneven transport of auxin down the axis. More auxin moves into the tissues that become the convex side of the curvature. Gravity seems to correct both unbalanced supply to and any unbalanced rates of its transport within the stem.

To demonstrate the adjustment of an unbalanced supply of auxin, we applied it, tagged with one $C^{14}$ atom, in a lateral strip near the decapitated tip of each defoliated Coleus axis in a series of tests. Forty plants were used in each of five experiments. Twenty of the plants were left erect to the normal action of gravity. The other twenty plants were turned on clinostats, usually for about 24 hours. These axes developed curvatures away from the side with the IAA-$C^{14}$. For both sets of plants, the treated tips and excess of radioactive auxin were removed. The curved axes of the plants on the clinostats were then split into equal weights of tissues on the convex and concave sides. The corresponding halves of the straight, erect axes were obtained in the same way.

The distribution of the auxin in each lot of tissues was determined from extracts made by grinding the diced tissues with chloroform and acid water and later with absolute alcohol. The amount of unchanged IAA plus its radioactive metabolic products was computed from counts of the radioactivity in the three extracts. The following table shows the results of these analyses and computations:

Table 1

| Exp. No. | Hrs. of Growth | Wt. in g. Clino | Wt. in g. Erect | Cpm. in 50 λ each of 3 extracts at .50 ml: On Clinostat IAA side | On Clinostat I/O | On Clinostat Opp. side | Erect to Gravity IAA side | Erect to Gravity I/O | Erect to Gravity Opp. side |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 42.0 | 12.72 | 10.61 | 26.0 | 1.340 | 19.4 | 25.7 | 1.015 | 25.3 |
| 2 | 18.0 | 9.04 | 8.65 | 100.2 | 1.411 | 71.0 | 107.4 | .976 | 110.0 |
| 3 | 24.0 | 7.57 | 10.07 | 132.7 | 1.375 | 96.5 | 110.2 | 1.005 | 109.7 |
| 4 | 23.5 | 11.06 | 11.32 | 106.1 | 1.428 | 74.3 | 218.0 | 1.048 | 208.0 |
| 5 | 24.0 | 11.17 | 11.10 | 414.1 | 1.448 | 285.9 | 387.9 | 1.010 | 384.2 |
| | | | | | 1.400±.030 | | | 1.011±.013 | |

These data show that the auxin from the lateral strip on one side of the stem's apex was transported equally to that side and the opposide side only when the axis stood erect to gravity. This evidence supports our hypothesis about the beneficial action of gravity and will be used in a technical paper as soon as certain related evidence can be obtained from new experiments with unbalanced rates of transport. Together with other evidence on file, we expect the new data will complete our proof that gravity is a significant factor in regulating auxin distribution within the axis of an erect plant.

Before the technical paper on this subject is offered for publication, it will be best to present evidence on a closely related phase of auxin transport — the downward transport of auxin by gravity in branches. This movement is suggested by the appearance of epinastic curvatures on clinostats, as described in one of our earlier publications (March, 1963). It has been reported and confirmed for Avena coleoptiles (in horizontal position) and reported for certain other plant organs but no work with comparable methods has been done for such transport in branches or the main stem of a plant.

Much of our work during the latter part of 1963 was directed to this point. We used Torenia for a few experiments with its small, numerous branches but Coleus proved to be a better test plant. Gravity curvatures were produced in its axes when young plants were laid horizontally. Similar curvatures were obtained in the branches of older plants, either by suspending them in the inverted position or by laying the plant on its side with the axis horizontal.

Data for the distribution of auxin within the curved stem tissues were obtained by the techniques of extraction and radioassay as outlined above for axis curvatures on a clinostat. From 15 to 20 plants were used for each test. The branches were defoliated and detopped; lanolin paste with 1% IAA-$C^{14}$ was applied as terminal caps to the stem tissue before placing the plants on their tips or sides; the curved tissues from negative geotropism were split into convex (=lower) and concave (=upper) halves, then diced and ground finely with chloroform, acid water and finally absolute ethanol to extract the auxin and auxin products from the two lots of tissues for each experiment.

The relative amounts of auxin within the upper and lower halves of the curvatures after a day or two of gravity effects were determined from the total radioactivity released by the $C^{14}$ in the extracts. The pertinent data are displayed in Table 2 much as it will be offered for publication in Plant Physiology as soon as certain other evidence can be prepared. The excess of radiocarbon in the lower tissues and hence of auxin movement to these tissues is not significantly different for the three sets of experiments. The ratio of auxin distribution after geotropic curvatures agrees well with that reported for other organs by earlier workers.

(see Table 2 on next page)

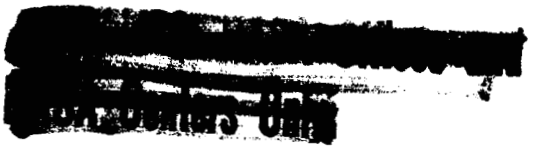

Table 2

RADIOACTIVITY FROM GRAVITY CURVATURES FED WITH IAA-2-$C^{14}$

| Exp. No. | Hrs. of growth | Wt. in grams | Coleus tissue | Cpm in 50 λ each of 3 conc. exts. Lower half | L/U | Upper half |
|---|---|---|---|---|---|---|
| 1 | 18.5 | 20.37 | Branch invert. | 444.8 | 1.464 | 303.9 |
| 2 | 18.5 | 17.25 | " " | 325.8 | 1.683 | 193.6 |
| 3 | 21.5 | 21.93 | " " | 367.0 | 1.247 | 294.2 |
| 4 | 17.0 | 15.57 | " " | 309.2 | 1.321 | 234.0 |
| 5 | 21.0 | 18.80 | " " | 243.7 | 1.479 | 164.8 |
| | | | | | M=1.439±.111 | |
| 6 | 21.0 | 11.86 | Axis horizon. | 15.7 | 1.702 | 9.2 |
| 7 | 21.0 | 12.39 | " " | 24.1 | 1.485 | 16.2 |
| 13 | 46.5 | 17.56 | " " | 59.5 | 1.574 | 37.8 |
| 15 | 47.0 | 20.06 | " " | 198.7 | 1.642 | 121.0 |
| 17 | 46.5 | 23.00 | " " | 141.4 | 1.209 | 116.9 |
| | | | | | M=1.522±.129 | |
| 10 | 44.0 | 16.75 | Branch on side | 432.7 | 1.441 | 300.2 |
| 11 | 47.0 | 35.48 | " " " | 1,684.2 | 1.696 | 993.2 |
| 12 | 45.5 | 26.05 | " " " | 452.5 | 1.367 | 331.1 |
| 14 | 44.0 | 31.81 | " " " | 1,011.4 | 1.731 | 584.3 |
| 16 | 45.5 | 45.16 | " " " | 936.4 | 1.677 | 558.4 |
| | | | | | M=1.582±.111 | |

To show that the radiocarbon was transported to the lower side in auxin form rather than in metabolic products, we must now complete chromatographic analyses of the chloroform extracts from these experiments. The first of these tests promises adequate proof of the point when the work has been completed. We hope that the editor of Plant Physiology will accept our manuscript based on this evidence for downward transport of auxin within the stem system of a mature, branched plant.

Most of our time and efforts during the past two months has been devoted to work for the Biosatellite Project. Our proposal for studying the orientation and rate of growth of seedlings at zero gravity has been scheduled for flight on Biosatellite A. Our proposal for use of a branched, leafy plant has been given back-up status and has not required our special attention recently.

As soon as the details of the pre-prototype plan for growing wheat seedlings in a small, closed system have been worked out, we expect to be able to carry on with our study of auxin transport and metabolism in combination with experiments to obtain the base line data for our biosatellite experiment. Our clinostats can be used for the wheat seedling work while we complete the biochemical and radioassay studies with extracts and certain lots of Coleus tissues being held in deep freeze. The biosatellite work would appear to be rather different but it has the great promise of checking out the validity of the clinostat as an instrument for simulating the actual absence of gravity in auxin studies.

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